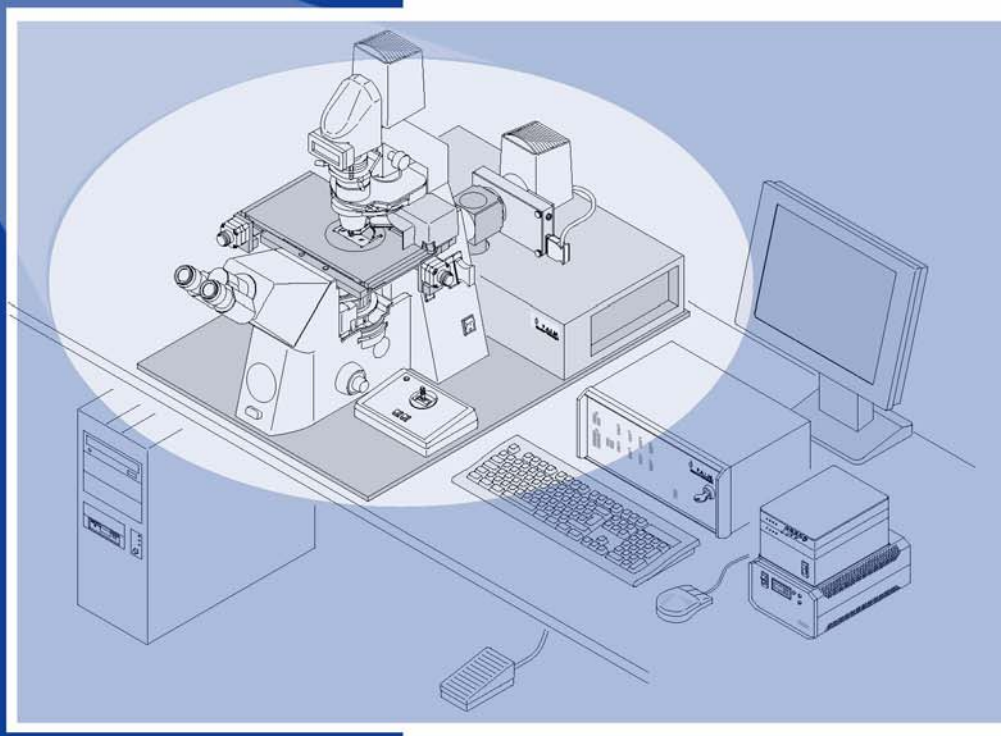
P.A.L.M.
Microlaser Technologies

PALM MicroBeam

User Manual



PALM MicroBeam
Version - 1105

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- Laser catapult technology (Laser Pressure Catapulting LPC^{pat})
  Patents: US 5,998,129, EP 879408 B1 and others.
- Three-dimensional laser beam positioning system
  Patents: US 5,689,109, EP 679325 B1 and others.
- Element List
  Patent: US 6,930,764
- Additional patents pending.

Regulatory Notice: PALM MicroLaser Systems are intended for research use only and are not approved for medical applications in the United States and Canada.

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1 Important Basic Information

Congratulations! With your new PALM MicroBeam from Carl Zeiss you obtained the State-of-the-Art Technology in microdissection and micromanipulation. The PALM MicroBeam is a high precision instrument, which allows to perform microdissection in nearly every kind of research as well as in routine examination with a higher throughput.

To meet a very high safety standard and to use all the benefits of your PALM MicroBeam you should read this user´s manual carefully and observe all instructions given in this manual. Please follow also the instructions for the single components of your PALM MicroBeam especially for the microscope, the fluorescence attachment (option), the video camera and the laser. P.A.L.M. Microlaser Technologies GmbH will not be responsible for mistakes and possible consequences caused by non-observance of the user´s manual.

Please consider also the manuals provided for your periphery devices!

1.1 Intended use of PALM MicroLaser Systems

The PALM MicroLaser family of products is a collection of state-of-the-art tools for precise, non-contact laser based microdissection and micromanipulation and consists of the PALM MicroBeam, the PALM MicroTweezers and as a combination of both, the PALM CombiSystem. It can be supplemented by different modules that can be adapted to the individual needs:

- Two different microscope stages are available (PALM RoboStage I and PALM RoboStage II) that can be equipped with different target holders for slides, petri-dishes, capillaries and even for work with serial sections (only RoboStage II).
- For the positioning of different collecting vessels there are available:
  PALM CapMover for fast positioning of collection vessels with highest precision.
  PALM RoboMover for work at higher troughput. This unique robotic collection device enables computer driven sampling of catapulted specimen from multiple spots into a variety of collecting devices.
- The fluorescence attachment expands the microscope to a fluorescence microscope.
- All functions can be controlled by the PALM RoboSoftware to facilitate operation and control of PALM MicroLaser Systems.

The lasers used are interfaced into a research microscope and focused through objectives with high numerical aperture to a minimum possible spot size. The effective laser energy is concentrated on this minute focal spot only and most biological objects are transparent for the applied laser wavelengths. It is therefore possible to work even inside a living cell without disturbing its viability.

Applications

PALM MicroLaser Systems are deployed in a wide array of research, biological and industrial applications. Our one-of-a-kind laser technology makes it possible for microscopic specimens to be positioned, microsurgically processed and captured with absolute precision and, unlike other systems, without risk of contamination due to our patented non-contact Laser Pressure Catapulting process lifting the desired objects into a vessel for further analysis only by the force of laser light.

It is intended for the collection of precisely defined cells (living or fixed), cell areas or subcellular particles from all kind of samples as they are used in pathology, oncology, neurology or are needed for proteomics and cytogenetics as well as genetic engineering or other fields of biological, medical or pharmaceutical research.

Microprocessing of non biological matter as for example latex beads or glass is also possible.

1.2 Legal Notes

P.A.L.M. Microlaser Technologies GmbH is not liable for damages resulting from the fact that this User Manual was not or only partly observed.

The warranty extinguishes in case the customer opens the laser housing or uses the device for other than the specified purposes.

If used parts are replaced by similar parts which are, however, not identical to those recommended by the manufacturer in this manual, P.A.L.M. Microlaser Technologies GmbH is only liable if the product supplied has already been faulty before the parts were replaced.

For details concerning the manufacturers warranty, please refer to the contractual conditions.

1.3 Conventions for this User Manual

Special markings on the left margin point out information you should observe by all means:

Warning
This sign warns you of possible most serious, irreversible injuries to persons even going as far as to death.

Caution
This sign signalizes possible moderate personal damage and possible damage of the device.

Notice
This sign signalizes that information is given demanding your special attention.

1.4 How You Can Contact Us

Business hours	9 h - 15.00 h CET, Mo - Fr
Phone	+49 (0) 81 58 99 71-0
Fax	+49 (0) 81 58 99 71-249
E-mail	Info@palm-microlaser.com

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www.zeiss.com

2 Installation

Your PALM MicroBeam is only to be installed by P.A.L.M. service engineers or their representatives. In case you want to move an already installed unit please contact P.A.L.M. Microlaser Technologies GmbH for assistance.

The PALM Laser Systems basically are maintenance-free.

In case of any malfunction of the device, please contact the PALM Service (see section 1.4, page 6).

3 Operating Room

In order to guarantee a correct function and comfortable work with your new PALM MicroBeam System, the following requirements have to be paid attention:

- The system should be installed in a room, which is adequately large (about 6 square meters) and well aired.
- There should be a free space available about 90cm above the table and all shelves, wall cupboards etc. should be removed.

The PALM RoboMover extends the dimension of a PALM System considerably to the left side. It exceeds the left side of the PALM MicroBeam base plate for 12 centimeters.

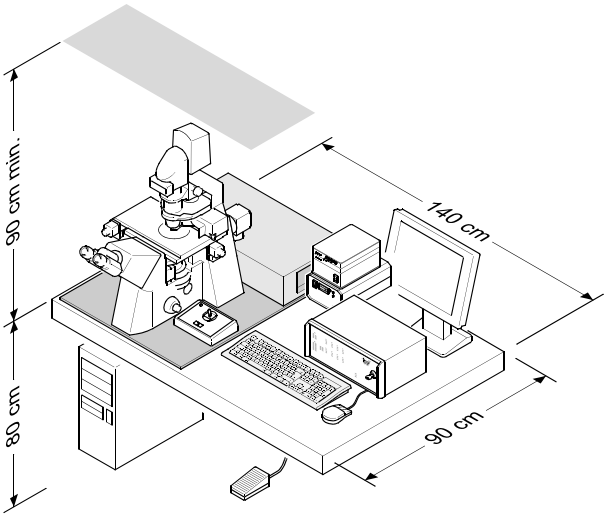


Figure 1 Dimensions

- Nature of the floor: low vibration (if not, special working table recommended) and even
- For a steel or granite table the load carrying capacity of the floor has to be > 400kg/m^2. Minimum space needed in total: 140x90cm
- For the aluminium plate a table measuring at least 120x90cm, on which the system will be set up, and with a load carrying capacity of the table of >90kg/m^2 is necessary.
- Power supply: 115/230VAC / 16VA safety fuse

Notice
Please consider also vibrations raising from your routine lab equipment!

3.1 Admissible Ambient Conditions

The device may be operated at the following ambient conditions:

- Ambient Temperature of the air: +15°C to +25°C (59°F to 77°F)
- Air humidity: 30% to 80%, not condensing.

4 Safety

4.1 What You Should Do in an Emergency Case

- Switch off the device immediately by turning the key switch in the OFF position (see Figure 4) and make sure that it cannot be switched on again.
- Unplug the multiple socket.
- Call the service staff in charge.

4.2 Observe the User Manual

This User Manual contains important information concerning the safe operation of the device. Only when strictly observing the User Manual, accidents can be avoided and a trouble-free operation of the device can be guaranteed. By observing the User Manual, downtimes and repair costs will be reduced and the service life of the device increased.

This User Manual must be stored close to the device at a place accessible and known to the staff, where it is available at any time.

This User Manual must be read, understood and observed in all points by the responsible and operating staff.

Please observe all safety instructions and other information given in the User Manual and the attached manuals of the accessories.

Caution
Any use other than the ones defined in this User Manual might lead to personal injuries or material damage which P.A.L.M. Microlaser Technologies GmbH is not liable for.

4.3 Laser Safety

The PALM MicroBeam is a laser device of laser protection class 1 M with no special safety requirements if operated according to the instructions given in this User Manual.

The accessible laser beam is safe for the unprotected eye when the laser casing is closed.

Built in is a Laser of class 3B. It bears a potential risk. A direct or reflected beam must not strike the unprotected eye (do not look directly into the beam).

In service case, the device has to be operated by the service staff or an authorized representative of P.A.L.M. Microlaser Technologies GmbH even with the laser casing open.

Warning
The housing of the Laser Interface may only be removed by the service staff or an authorized representative of P.A.L.M. Microlaser Technologies GmbH.

Warning
Please note: wavelength is invisible for the human eye (λ = 337nm resp. λ = 355nm).

4.4 Safety Markings and Type Plate

Make sure that the following safety markings and the type plate exist and are legible. Replace missing or illegible safety markings or type plate.

Caution
The safety markings on the device must always be clearly visible and legible and must strictly be observed.

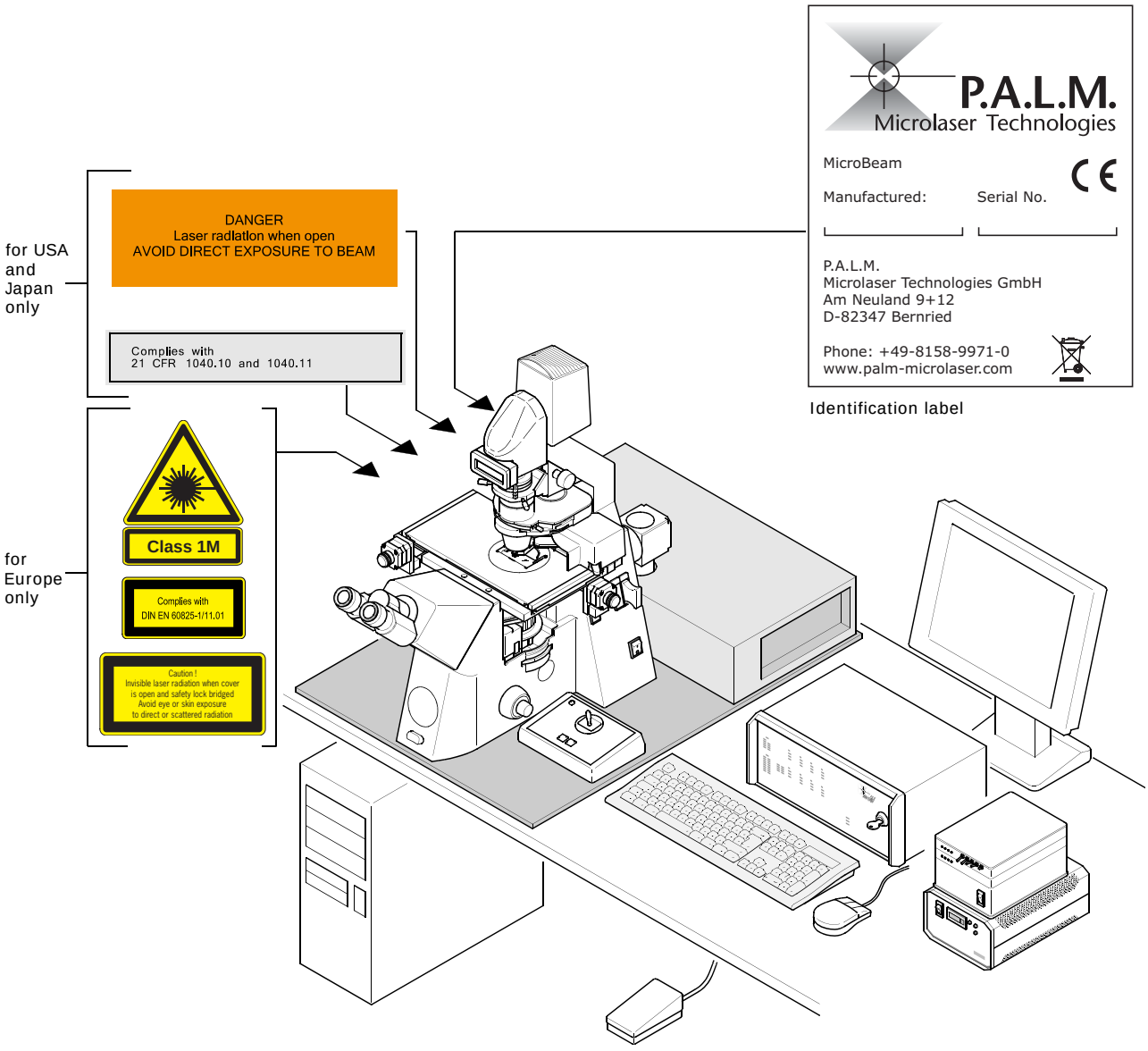


Figure 2 Safety Markings and Type Plate

4.5 Qualification of the Staff

The device shall only be operated by trained and specially instructed staff having the legal minimum age and having carefully studied this User Manual.

Staff in the course of being taught, trained, instructed or being in a general education is only allowed to work with the device under permanent survey of an experienced person.

4.6 Points that must strictly be observed

Caution
Service Works on the laser may only be performed by P.A.L.M. Microlaser Technologies GmbH or its representative.

When service is needed, please contact the P.A.L.M. Microlaser Technologies GmbH Service.

- Protective equipment may not be circumvented, removed or made ineffective, at any rate.
- All requirements regarding the condition of the device, the qualification and behavior of the staff, set forth in the User Manual have to be met.
- Make sure that unauthorized persons cannot use the device.
- The device may not be operated or maintained without wearing the prescribed personal protective equipment.
  Please find the respective instructions in the relevant chapters in this manual.
- Persons under the influence of alcohol, other drugs or substances that affect the ability to react are not allowed to operate the device.
- The device may only be used in a technically faultless condition. In case of troubles or problems with the power supply and/or damage of the electric equipment, the device has to be shut off immediately.
  Any malfunctions or damage indicated by the system or detected otherwise must immediately be notified and removed.
- The device may not be modified.
- Protective equipment (e. g. protective filters or covers of laser beam outlets) may not be dismantled.
- The device may only be operated if the entire protective equipment is perfectly working.
- The application conditions and performance data defined in this User Manual may not be altered. For Technical Data see section 10, page 32.

4.7 Pay Attention to Remaining Risks and Preventive Measures

4.7.1 Remaining Risks and Protective Measures – Laser

- Remaining risks
  - The laser works with invisible light.
    When the casing is open, the laser pertains to the laser protection class 3B. The high power radiation leads to dimmed sight of the eye.
  - The casing must always kept closed and locked and may only be opened by the service staff of P.A.L.M. Microlaser Technologies GmbH.
  - Unintentionally mirrored reflections must be avoided by a risk-conscious behavior of the staff.
- Protective measures taken by the manufacturer
  - On the microscope lighting arm and the condenser (if it can be swiveled out of the beam paths) a safety switch is installed that shuts off the laser as soon as the lighting arm is swiveled.
  - The travel paths of the laser beams as well as those of stage and CapMover are equipped with end switches to avoid damage.

4.7.2 Remaining Risks – Electricity

- Before cleaning current-carrying parts, make sure that the power has been switched off.

Even after switching off the electric energy supply, life-threatening voltage might occur in areas marked with an electric flash as well as in the following supply systems/dangerous areas:

- Process computer
- DC power unit
- Laser, fan
- Stepping motor control
- a.c. network feed lines.

4.7.3 Remaining Risks – Infectious Material

Danger of infectious particles.

Works on infectious materials (e.g. viruses) must be carried out under a sterile protective cover. When working with infectious material, the respectively applicable safety regulations must be observed.

Clean the working area of the microscope after use, if required. The cleaning agent should neither be abrasive nor strongly basic or acid, as this would attack the protective coating.

4.7.4 Remaining Risks – EMC

Regarding interference resistance, the device meets the protection requirements according to DIN EN 61000-6-2.

Regarding interference emission, the device meets the protection requirements according to DIN EN 55011.

Do not operate any devices emitting high-frequency radiation in the same room, where the PALM MicroBeam is placed.

4.7.5 Remaining Risks – Fire Extinction

To extinguish fire, use sand or carbon dioxide. Never ever use water because of the electric dangers.

4.8 Disposal

4.8.1 How to Ensure a Safe and Ecological Disposal

Dispose the following materials in an environmentally friendly way:

- Microscope lamps with incorporated bulb or individual mercury vapor short arc lamps as hazardous waste
- Laser as electronic scrap
- Electronics as electronic scrap
- Cables as reusable material.

4.8.2 For EU states only

PALM Microlaser devices are produced according to the EU guidelines 2002/95/EG (RoHS) and 2002/96/EG (WEEE), as far as these are applicable.

We have installed a process for disposal and recycling within the member states of the guidelines mentioned above.

Please contact your relevant Carl Zeiss Service resp. sales organization to learn about the details for the disposal or recycling of your device.

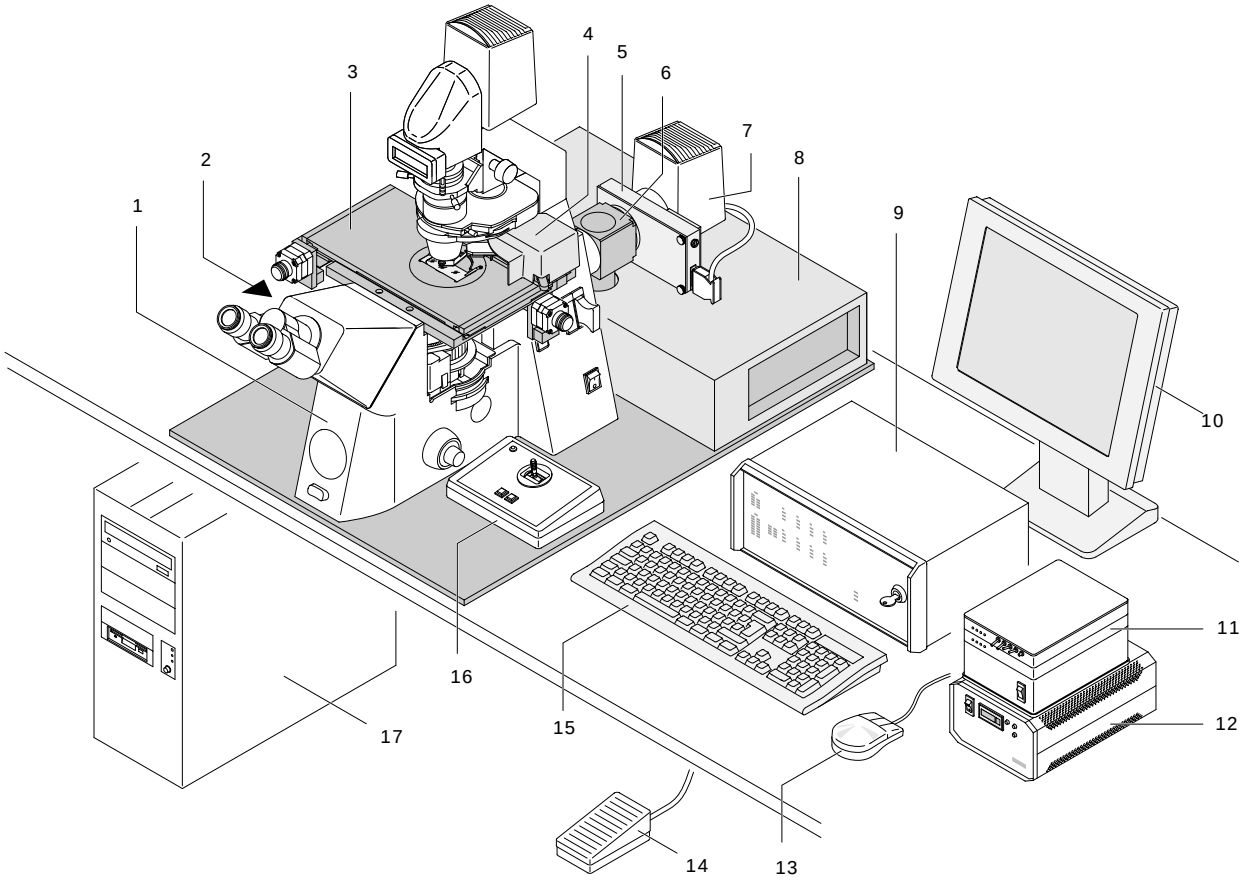
The product must not be disposed of the domestic waste or be given to the municipal disposal collection. In case of resale, the seller is committed to point out to the buyer that the product has to be disposed according to the guidelines.

5 The PALM MicroBeam - Survey

Modular Survey

The PALM MicroBeam Device consists of the parts shown in Figure 3.

These parts and their function are described in detail in the following chapters.



1 microscope (see section 5.4, page 15)
2 CCD video camera (see section 5.9, page 20)
3 microscope stage (see section 5.6, page 17)
4 CapMover (option) (see section 5.7, page 18)
5 filter wheel (option)
6 microscope adapter (see section 5.3, page 14)
7 fluorescence illumination (option) (see section 5.5, page 16)
8 Laser Interface and laser (see section 5.2, page 13)
9 control unit (see section 5.1, page 13)
10 monitor (see section 5.9, page 20)
11 control unit (filter wheel)
12 power supply (fluo illum.)
13 mouse
14 foot stepper (see section 5.11, page 20)
15 keyboard
16 joystick CapMover (option)
17 computer (see section 5.10, page 20)

Figure 3 Survey of the PALM MicroBeam

5.1 PALM ControlUnit

The PALM ControlUnit is the main device, which runs and controls the movement of the PALM RoboStage, energy- and focus unit and the laser. The key switch also operates the mains for the single components of your system. So you can switch on/off the whole system with this key switch. If you want to run single components as the computer for example, it is possible to connect these components directly to the mains.

The actual status of the PALM ControlUnit can be observed by LED´s on the front panel.

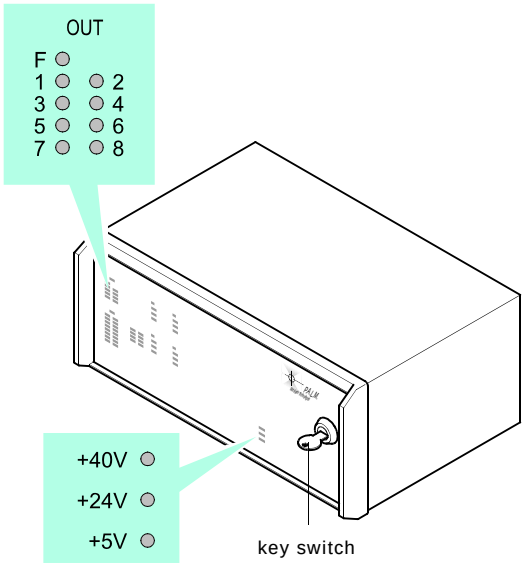


Figure 4 PALM ControlUnit

After switching on the system with the key switch, the three diodes for the voltage ranges +40V, +24V and +5V should be illuminated and the green LED "2" should flash every second.

Notice

Shortly after switching on the PALMControlUnit the red LED "F" turns off automatically. Otherwise switch off the ControlUnit and after 10 seconds switch it on again. Should the LED not turn off please contact P.A.L.M. Microlaser Technologies GmbH.

The type of ControlUnit may differ depending on the individual device configuration.

5.2 Laser Interface

The Laser Interface is a one-box device, which houses the laser and all the necessary optics to guide the laser into the microscope, to bring the laser focus coincide with the optical focus of the microscope at the object plane and to adjust the laser energy.

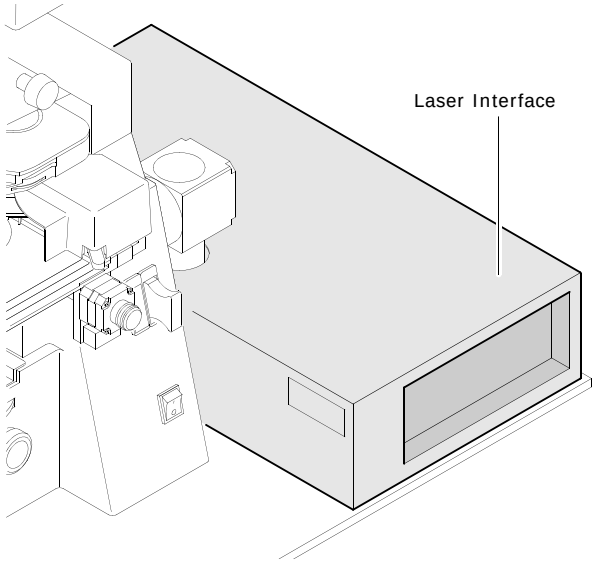


Figure 5 Laser Interface

This is a prerequisite for precise laser micromanipulation with highest precision.

Furthermore, the laser position stays stable, which allows to routinely work with the system without time consuming search for the laser beam focus.

Notice

The adjustment of laser focus and energy settings are done by the user (see chapter 6.5, also observe PALM RoboSoftware Manual).

Caution

Do not open the housing of the Laser Interface. Invisible radiation of a laser class 3B might be guided unprotected to your eyes!

Laser

The PALM MicroBeam works with a pulsed N-laser (wavelength: 337nm) or with a frequency tripled solid state Laser (FTSS, wavelength: 355nm) of a high beam quality, which is coupled via the epifluorescence path (see Figure 7, Pos. 7) into a research microscope and focused through the objective. Depending on the applied objective the laser focus can reach less than one micrometer in diameter. In theory, the high beam quality of the laser allows to focus the laser beam to its diffrac-

tion limit. However, the cutting width is dependent on

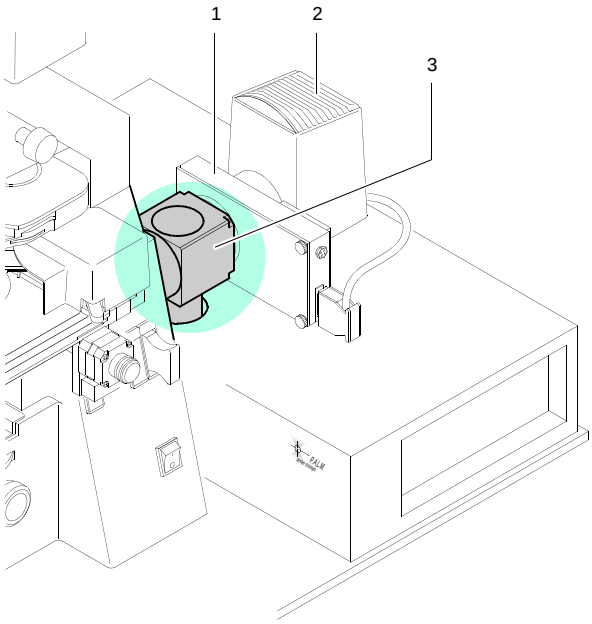
- correct laser focus settings,
- optimal laser energy settings,
- the numerical aperture of the focusing objective and
- the absorption behavior of the specimen.

Notice
P.A.L.M. provides factory settings for each objective. The fine tuning of laser focus and energy setting is done by the user (please see section 6.5, page 23) and is dependent on the focusing objective and the kind of material you want to handle.

For cutting, the stage is moved and not the laser beam itself.

5.3 Microscope Adapter

The microscope adapter connects the laser to the microscope. The laser beam is guided into the microscope and deflected into the objective.



1 filter wheel, filter holder with shutter (option)
2 fluorescence illumination lamp (option)
3 microscope adapter

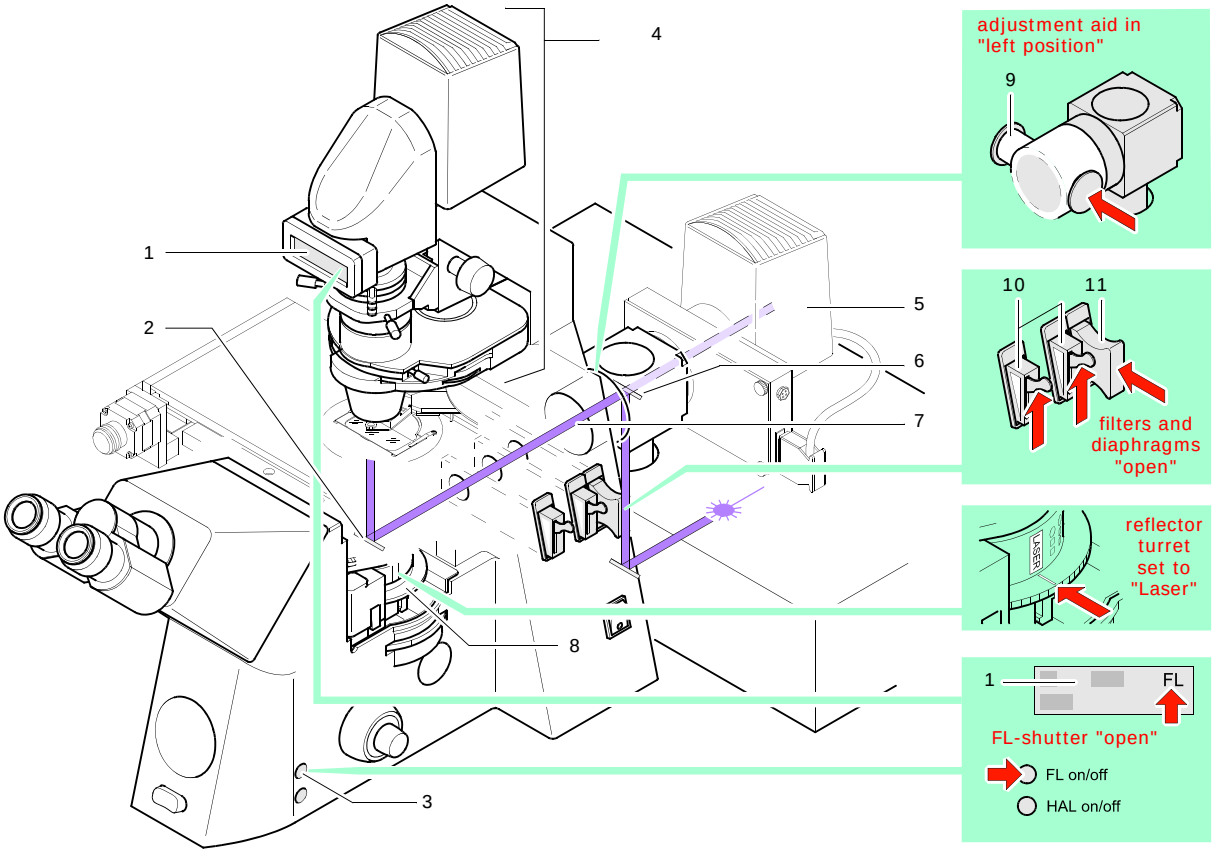
Figure 6 Microscope adapter

The especially designed beam splitter (see Figure 7, Pos. 2) allows to connect the fluorescence illumination lamp and the filter wheel to the system for simultaneous usage of fluorescence illumination with laser application.

Notice
Due to the high power laser, for simultaneous fluorescence illumination especially designed beam splitters have to be used. For more information please contact P.A.L.M. Microlaser Technologies GmbH.

Caution
Do not touch or remove the screws on the microscope adapter. The alignment of the system would be destroyed and if the mirror is removed, invisible radiation of a laser class 3B might be guided unprotected to your eyes!

5.4 Microscope Zeiss Axiovert 200 / 200M; preparation for laser operation



1 display
2 special coated beam splitter
3 button for fluorescence shutter
4 illumination arm
5 fluorescence attachment (option)
6 microscope adapter
7 epifluorescence channel
8 reflector turret
9 adjustment aid
10 two diaphragms
11 filter slider

Figure 7 Survey of the microscope

The high optical and mechanical quality Zeiss inverse microscope Axiovert 200 resp. 200M is part of the PALM MicroBeam. This microscope can also be used as a normal research microscope. Please observe the Axiovert 200 resp. 200M user´s manual.

The laser beam is guided through the microscope adapter (see section 5.3, page 14) into the epifluorescence channel of the microscope. It is reflected by a special coated beam splitter in the reflector turret and focused by the objective.

It is necessary to observe the following instructions, otherwise the laser beam might be blocked and performing microdissection will not be possible:

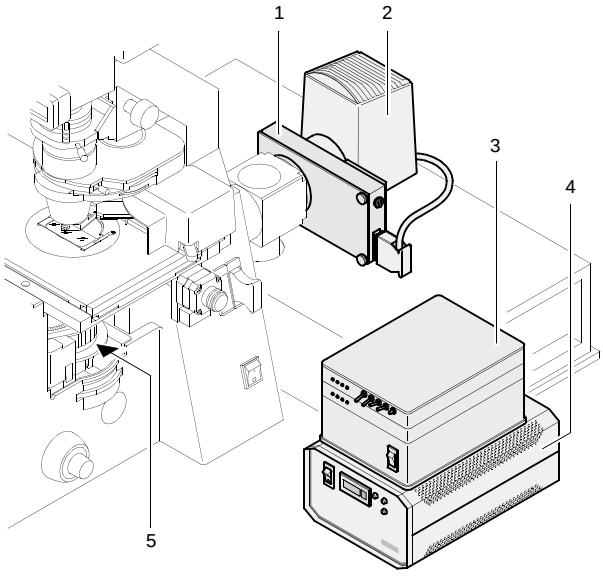
- The electromagnetic fluorescence shutter in the microscope has to be opened. This can be done with the button "FL on/off". When the shutter is open, the sign "FL" appears on the display of the microscope.
- The reflector turret has to be in the position in which the beam splitter reflects the laser beam. This position is labeled with "1 Laser" (see indication plates at the front of the microscope), accordingly the reflector turret has to be set on position "1"). In case of installed fluorescence attachment (option), it is possible to use also the installed beam splitters of the fluorescence filter sets (only in case of advanced filter sets).
- The two diaphragms in the epifluorescence channel must be open.
- The adjustment aid for the fluorescence illumination has to be in the left position, otherwise the laser beam will be blocked.
- Do not insert any filters in the filterslider in the epifluorescence channel. They might block the laser beam, therefore put it to free passage (rightmost position).

Notice

- The magnification lens is not in the laser beam path. So if the magnification selector knob is pulled out, there is an additional magnification by 1.6x in the light path to the video camera but not in the laser beam path. So the laser cuts and catapults not the lines and points you marked on the screen.
- If the illumination arm of the microscope is pushed back, the laser will be switched off immediately (Interlock). This is a security device, which protects you from looking into the laser beam.
  If you use a FTSS Laser, you will have to reactive the laser emission by pressing the laser ON/OFF button.

5.5 Fluorescence attachment

The fluorescence attachment expands the microscope to a fluorescence microscope.



1 filter wheel, filter holder with shutter
2 HBO lamp or X-Cite
3 controller for filter wheel
4 power supply
5 especially designed beam splitter

Figure 8 fluorescence attachment

The filter wheel is accessed by the PALM RoboSoftware. It is mounted outside of the optical path of the laser.

To change the lamp please refer to microscope manual.

For the correct settings of the power supply and the controller for the filter please consult the PALM RoboSoftware Manual.

For detailed information about operating the microscope with fluorescence illumination please see section 7, page 26.

Depending on specific customer requirements, P.A.L.M. offers two different possibilities of fluorescence attachment:

Basic Fluorescence Attachment

(for PALM MicroBeam with Zeiss Axiovert 200/ 200M)

Simultaneous fluorescence observation and laser functions are not possible, resp. wanted. Depending on the type of microscope, the handling of the filter revolver for filter change and cutting position is done manually.

Fluorescence Hardware components:

- HBO lamp or X-Cite
- PALM FluorescenceShutter (standard)
- Any Basic Fluorescence Filter Set

The basic fluorescence attachment is suitable for fixed samples only.

Advanced Fluorescence Attachment

(for PALM MicroBeam and PALM CombiSystem with Zeiss Axiovert 200 / 200M):

Simultaneous laser functions and fluorescence illumination are provided.

Fluorescence Hardware components:

- HBO lamp or X-Cite
- Motorized filter wheel
- Selected Advanced Fluorescence Filter Sets

Depending on the type of microscope (motorized or manually), shutter and filter wheel there is a different adjustment necessary.

Notice
If you use a Zeiss ApoTome, Advanced Fluorescence is not possible.

5.6 PALM RoboStage

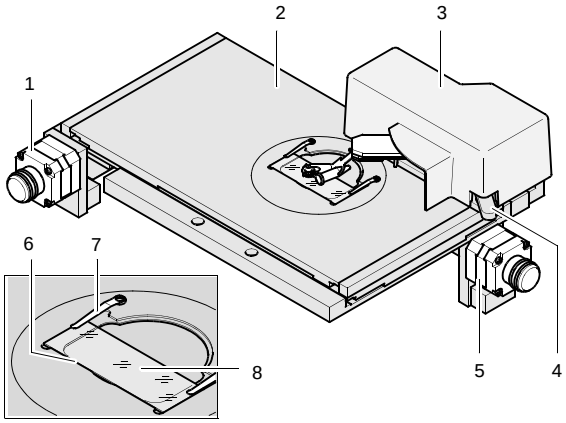
P.A.L.M. has developed the PALM RoboStage I and II. The computer controlled PALM RoboStage is a motorized stage especially designed for high-precision laser micromanipulation in sub-micrometer range. Its movement is carried out by two stepping motors at a speed of only a few µ-meters up to several mm per sec. and displayed largely vibration-free between 1µm and approx. 60 mm. The stepping motors are controlled by two encoders that are affixed below the stage. The maximum traverse path is 40 mm in y-direction and 60 mm in x-direction for the PALM RoboStage I or 76 mm in y-direction and 114 mm in x-direction for the PALM RoboStage II.

Caution

- Do not move the stage manually!
- Do not touch the moving stage!
- Avoid contaminating the guide of the stage with oil or chemicals. Avoid any kind of mechanical stress, e.g. putting heavy parts on it or leaning against, as it is a precision instrument.

Notice
Do not clean the stage with alcohol!

5.6.1 PALM RoboStage I



1 stepping motor Y-direction
2 microscope table board
3 CapMover
4 lever for cap height adjustment
5 stepping motor X-direction
6 holding frame
7 snap-on clip
8 specimen slide

Figure 9 PALM RoboStage I

Several special functions are integrated to speed up routine work.

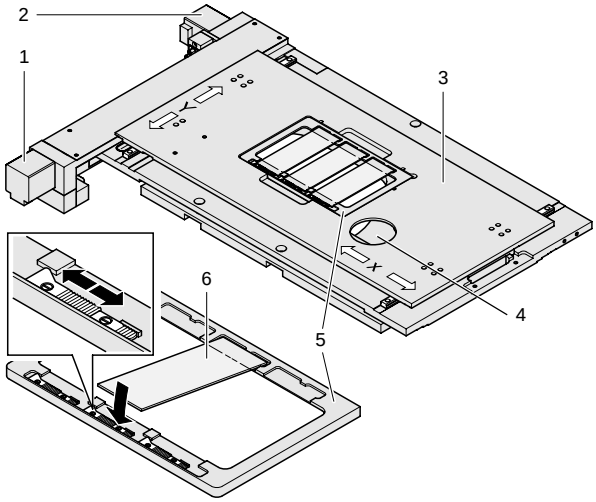
In the PALM RoboStage I there is a holding frame into which the specimen slides or capillaries can be clamped. Optional there is a double slide holder available.

Both transversal sides of the object slide are held by a snap-on clip.

Notice
At the longitudinal side of the holding frame there is a special rim. Please make sure that the longitudinal side of the slide rests on this rim entirely. This will reduce the risks that 0.17 mm slides will bend by the adhesion force of the immersion oil.

The PALM RoboStage I will be moved by means of the PALM RoboSoftware.

5.6.2 PALM RoboStage II



1 stepping motor Y-direction
2 stepping motor X-direction
3 microscope table board
4 Check Point
5 3-SlideHolder
6 specimen slide

Figure 10 PALM RoboStage II

In addition RoboStage II fulfills its specification in high throughput mode. Up to 3 microscope slides can be investigated at the same time, which is useful especially for serial sections. Also different stage inserts like petridish holders for living cells, capillaries and microtiter plates can be inserted by using P.A.L.M. designed stage inserts (ask P.A.L.M. Microlaser Technologies GmbH for details).

For easy insert change, a so called "Load position" is defined. In this position the stage insert or microtiterplate can be withdrawn powerless and any kind of holder can be inserted.

A separate "check position" located on the right side of the Stage II allows for checking the catapulted and captured samples with all collection devices.

Inserting a 3-SlideHolder into Stage II

The surface of the stage is even and not narrowed down by engines or spindles. There are two 3-SlideHolders available (1mm, black anodized and 0.17 mm, achromatic anodized) that can be used only in combination with PALM RoboStage II. The slides have to meet the demands of DIN ISO 8037-1, this means 25-26 mm width and 75-76 mm length.

PALM RoboSoftware 3.0 or a higher level is required.

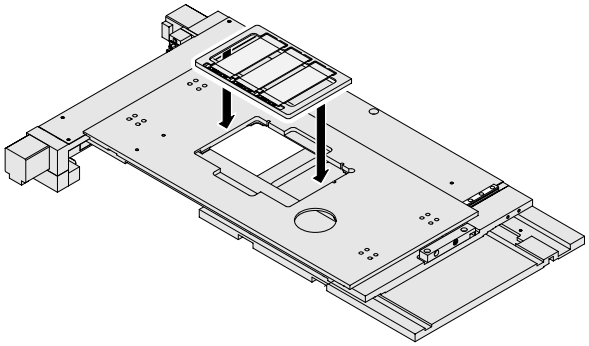


Figure 11 Inserting a 3-SlideHolder into Stage II

- For loading start the PALM RoboSoftware and wait until the referencing of the stage has finished and it has reached the central position again.
- Press the button "Loadposition" and wait until the loadposition is reached.
- Withdraw a possibly assembled holder and insert the 3-SlideHolder into the provided opening. The sharp edge has to be positioned at the rear right. Due to this edge the holder can only be inserted in one direction.
- In order to return to the work position press the button "Loadposition" again.

The software now asks you to select the correct holder.

- Choose "PALM Robo" in the menu "Setup".
- Select "Configuration" and click on "3-SlideHolder".

Notice
The objectives have to be focused on the sample or lower to avoid a collision between the objective and the stage.

Insertion of the slides in the 3-SlideHolder

- Choose the appropriate slides.
- Push the slider (the metal clamp on the lower side of the Holder) to the left and insert the slide.
- Make sure that the slide fits exactly into the three edges.
- Release the slider carefully.

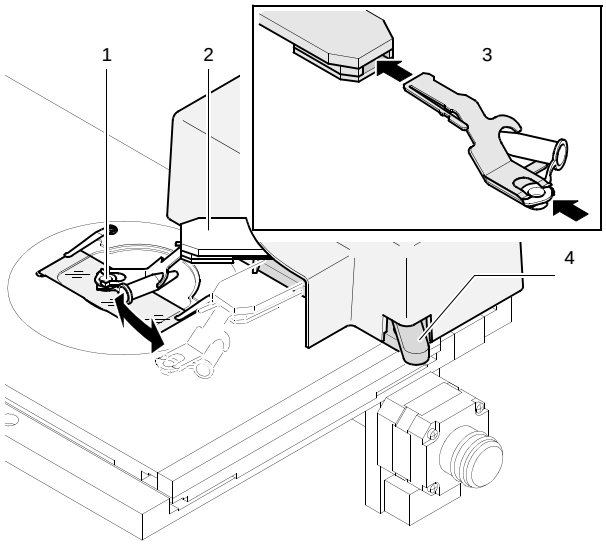
Notice

- When you change from one slide to another you will have to focus manually.
- If you use an 100x oil objective, lower the stage when changing from one slide to another.

5.7 PALM CapMover

The motorized PALM CapMover was developed for fast positioning with highest precision and quick exchange of the collection device for sample collection. There are different collection devices and collection device holders for up to 8 caps or single tubes available. This way, the cap is positioned and moved via joystick to check for the catapulted specimen. Please contact P.A.L.M. Microlaser Technologies GmbH for further information.

Caution
If any vessels (e.g. Petri Dishes) instead of specimen slides are used make sure that the cap resp. tube holder is in the upper position when moved in or out. Otherwise the PALM CapMover may be damaged.



1 cap
2 CapMover
3 CapHolder
4 lever for cap height adjustment

Figure 12 CapMover

Control Apparatus

At the front of the control apparatus there are two buttons, a joystick and an LED.

The LED essentially indicates the operational readiness. In the event of an error or a circuit overload, this LED will extinguish. In this case, disconnect the apparatus from the power supply and ascertain the cause of the error. For example check if the traverse path of the PALM CapMover is blocked. You should not switch on the control apparatus until after having done this.

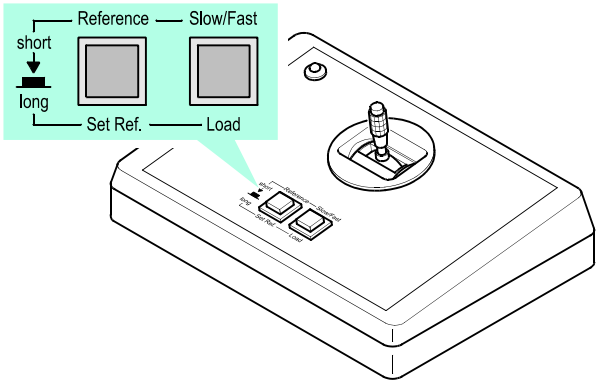


Figure 13 Control Apparatus

Brief extinguishing of the LED also indicates a change of speed, as well as the saving of a new reference position.

The joystick serves to move the PALM CapMover arm - and consequently the cap or tube - in two dimensions.

The buttons have the following functions:

Left button

If the button is pressed briefly, the PALM CapMover arm moves to the reference position that is currently saved. By pressing the button for a second time the arm moves back to the last position it was moved to.
If the arm has been moved from the reference position with the joystick, then this position is accepted as the last targeted position.

If the button is pressed for a while, then the current position is saved as the new reference position. The LED will extinguish briefly as a confirmation signal.

Right button

If the button is pressed briefly, this will switch between the 'fast' and 'slow' speed ranges. The LED will extinguish as a confirmation signal with each change of the speed range.
The faster speed range is suitable for examination at a low optical magnification factor, whilst the slower speed range is useful in the case of high magnification.

Prolonged pressing of the button will cause the arm to move outside of the objective area - for example in order to take up a new cap.
Speed change, joystick and the functions of the left button are deactivated in this position.
Renewed activation of the right button will cause the arm to be retrieved and the cap to be moved to the saved reference position.

5.8 PALM RoboMover

The PALM RoboMover is the state of the art device for modern cell harvesting and sorting of different kinds of microdissected specimen at higher throughput. It enables you to automatically position collecting devices above your specimen. There are various collectors available containing one or several caps or tubes or even collectors for microtiterplate formate. All functions are controlled by the PALM RoboSoftware.

For more details see the PALM RoboMover Manual.

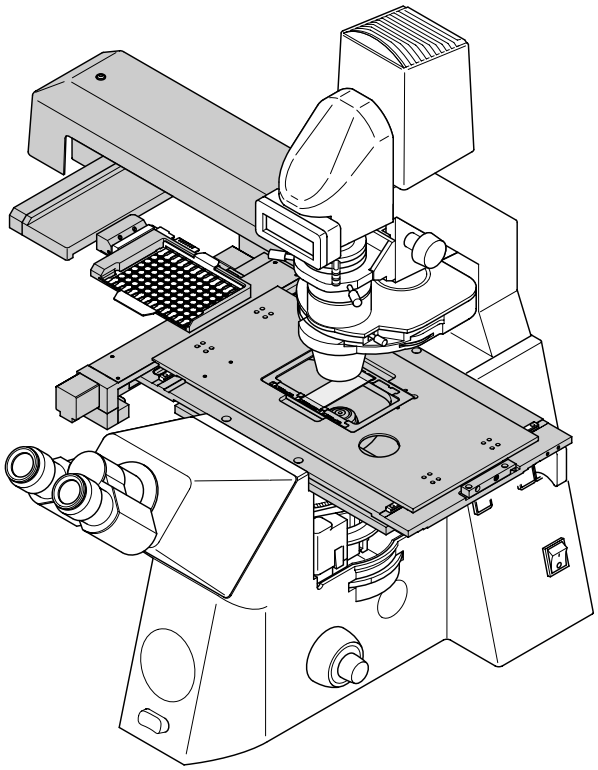


Figure 13 PALM MicroBeam with PALM RoboMover

5.9 CCD Video Camera and Monitor

Pictures taken by a CCD camera are transmitted (directly) to the computer via a frame grabber.

The video transferred microscope image is displayed in real time on the monitor screen, which allows to simultaneously observe the microscope image with stage or manipulator control functions.

You can store the transferred images on the hard disk of the computer or on CD.

The camera is preset by P.A.L.M. Microlaser Technologies GmbH.

For changing camera parameters please observe the manual of the camera.

Notice

- Depending of the Camera Adapter, the computer monitor only shows a section of the area visible in the ocular.
- There are several camera ports available to integrate up to 5 cameras.

Caution

The camera is aligned in parallel to the stage. Don't turn or remove the camera in any way, otherwise the calibration of the system is destructed and the laser light might leak out from the camera port.

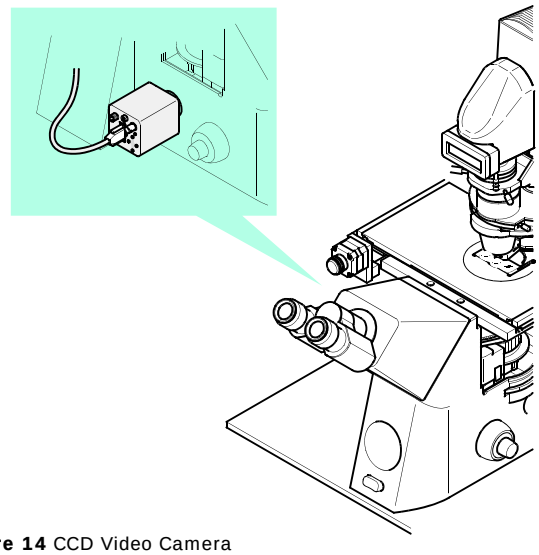


Figure 14 CCD Video Camera

5.10 Computer

The system is equipped with a state of the art computer. The computer itself is provided with a CD-burner and with an image handling software, for easy image selection and editing, as well as archiving. With the integrated network card you have the possibility to connect your computer with your local network. If you want to connect it, please contact your local area network administrator.

Notice

It is not allowed to change or modify software or hardware components on the computer. P.A.L.M. Microlaser Technologies GmbH is not responsible for any damage resulting from disobeying these points. Any modification of the computer hard- or software underlies to the prior written consent of P.A.L.M. In any case, P.A.L.M. is not liable for any loss due to any kind of computer virus.

5.11 Foot stepper

The foot stepper is used to activate the laser cutting function manually. This option is only available in stage mode (please refer to the PALM RoboSoftware Manual).

5.12 ApoTome

ApoTome, the innovative insert module from Carl Zeiss, allows the very fast production of extremely high-quality optical sections through fluorescence-labeled biological specimens – with two- and three-dimensional visualization. ApoTome offers better image quality, contrast and resolution.

Notice

If you use a Zeiss ApoTome, Advanced Fluorescence is not possible.

Caution

When the laser runs, make sure that the grid of the ApoTome is not within the optical path, and the position of the ApoTome must not be changed. Otherwise the transmission grid may be destroyed.

To avoid simultaneous laser function and integration of the transmission grid, a safety switch can be integrated into the system. Ask P.A.L.M. Microlaser Technologies GmbH for details.

6 How to Prepare the PALM MicroBeam for the Application

6.1 How to switch On/Off the Device

6.1.1 Switch On the Device

- Set the key switch of the ControlUnit to ON and switch on the computer (for details see section 5.1, page 13)

  The PALM RoboSoftware will be loaded automatically. If not, please refer to the PALM RoboSoftware Manual how to start it.

Notice
If the red system LED "F" of the control unit (see Figure 4) does not turn off automatically, please switch off the ControlUnit and after 10 seconds switch it on again. Should the LED not turn off please contact P.A.L.M. Microlaser Technologies GmbH.

6.1.2 Switch Off the Device

- Exit WINDOWS.
- Switch off the ControlUnit.
- Switch off the monitor.

6.2 CCD Video Camera Adapter

Caution
Do not touch the camera! If camera position is changed, new calibration is necessary (please refer to the PALM RoboSoftware Manual how to calibrate your system).

Notice
Depending of the Camera Adapter, the computer monitor only shows a section of the area visible in the ocular.

6.3 Check of the laser beam path in the Microscope

The laser is coupled into the microscope via the epifluorescence beam path.

Prior to the use of the laser, make sure

- that the aperture of the iris diaphragm in the epifluorescence beam path is completely open (see Figure 7, Pos. 10),
- that a possibly existing filter slide is set to free passage (see Figure 7, Pos. 11),
- that the reflector turret under the objective is switched into the correct position called "laser" (see Figure 7, Pos. 8)
- that the Fluorescence shutter in the reflector turret of the microscope is open: "FL" appears in the display. (see Figure 7, Pos. 8)
- adjustment aid is in left position (see Figure 7, Pos. 9).

Caution
Only use the objectives that are authorised by P.A.L.M. Microlaser Technologies GmbH for laser operation.

Objectives designed for oil immersion or water immersion must be used with the respective immersion medium, otherwise the objectives might be damaged.

Caution

- Do not turn the objective turret during simultaneous laser operation.
- Never use the laser without an objective in the appropriate position.
- Never insert reflecting objects in the laser beam path.

6.4 FTSS Laser

If you use a PALM MicroBeam system with FTSS Laser, the laser is operated via an additional control box (Figure 15).

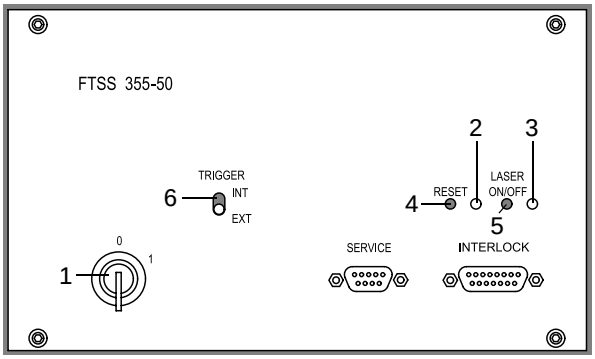


Figure 15 Control Box

6.4.1 How to switch On/Off the Laser

Switch On the Laser

- Press the red main power switch on the backside of the Laser Controller and turn the key switch (1) on the front panel into position "1". Only the left LED (2) will light up yellow, indicating that a supply voltage is present.

After a warm-up period of approx. 5 minutes the left LED (2) and the right LED (3) will light up green and the laser is ready for operation and can be activated.

Notice
If the green LED (3) is not glowing, the laser cannot be started and must be reset with the reset button (4) or by turning the key switch off and on again.

- To activate laser emission, press the LASER ON/OFF button (5).
  The green LED (3) will change color and lights up red. After a delay of approx. 1 second, the laser emission starts.

Notice
If laser emission does not occur, check that the Trigger switch (6) is positioned on "Ext".

The laser works with an output pulse energy regulation. After a total warmup period of less than 10 minutes the laser parameters will be within the specifications.

Interlock

When changing your samples on the microscope stage, you should put the microscope illumination arm back thus activating the laser safety interlock. The laser emission will stop immediately and only the left LED (2) will light up yellow.

To reactivate the laser emission, press the LASER ON/OFF button (5). After approx. 1 second the right LED (3) will light up red.

Switch Off the Laser

- To deactivate the laser emission, press the LASER ON/OFF button (5). The red LED (3) will extinguish.
- To finish work with the laser system, turn the key switch (1) into position "0" and switch off the main power supply.

6.5 Laser energy and focus

To be able to precisely cut the material and reliably catapult it, the laser focus has to be set accurately to optimal values under various conditions.

The diameter of the cuts is dependent on

- the numerical aperture of the objective used
- the position of the laser focus with regard to the microscope focus
- the applied laser energy
- and on the absorption properties of the material to be cut.

The ablation occurs within the area of the laser focus (please see figure 16a). The smallest available cutting size can only be achieved within the center of the laser focus using as little energy as possible.

The catapulting is performed by placing the laser focus underneath the specimen to be catapulted (please see figure 16b)

The laser beam is guided through the epifluorescence channel of the microscope and focused through the objective. Therefore the energy and the size of the laser focus depend on the applied objective.

Generally the following is valid: The higher the magnification and the aperture of the objective, the smaller the focus of the laser and the higher the energy density.

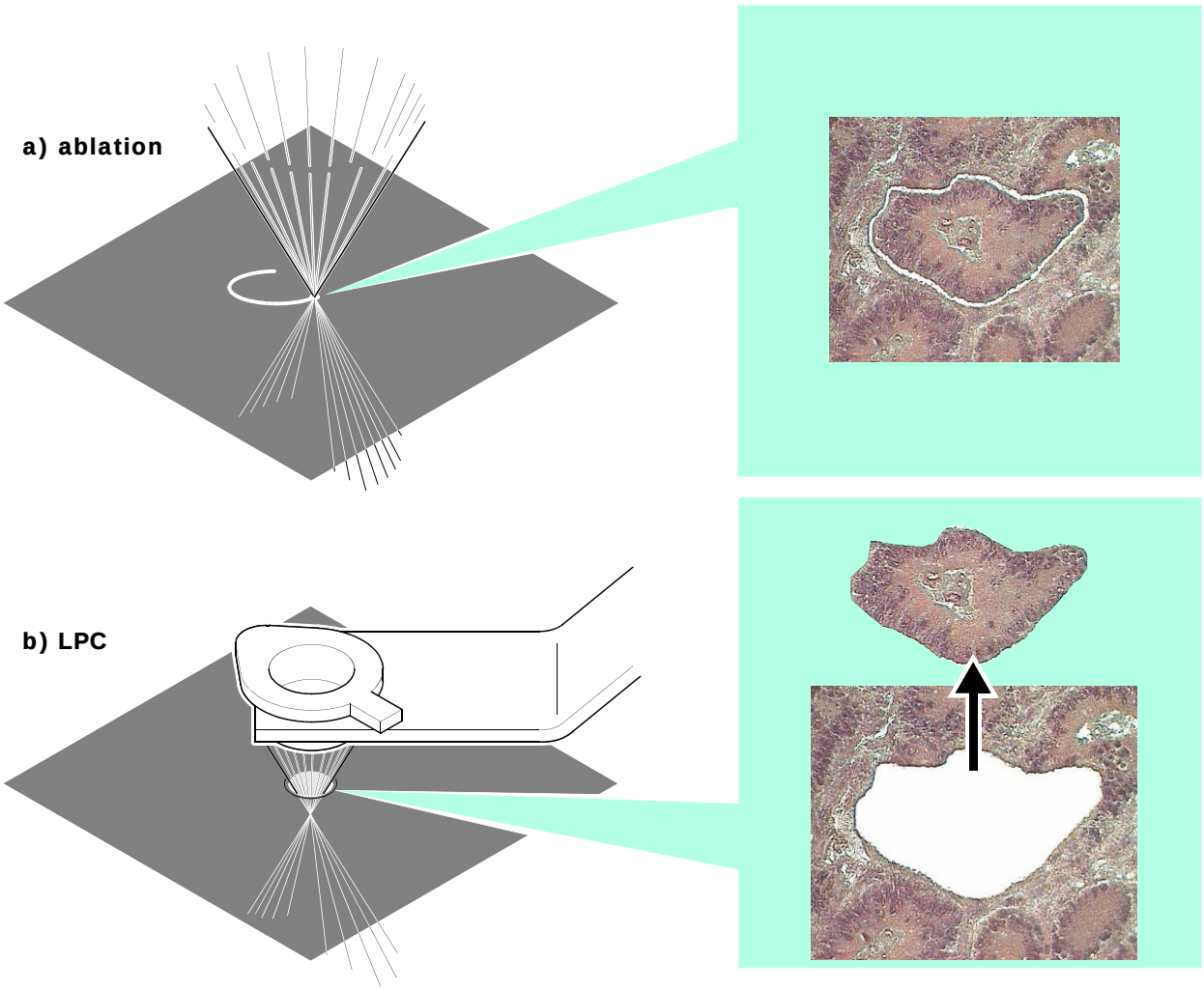


Figure 16 Position of the laser focus, when ablating (a) or catapulting (b)

6.5.1 Laser Energy Adjustment

The laser emits a fixed laser energy. For precise laser micromanipulation a precise laser energy adjustment is required.

A neutral density filter is mounted into the laser beam path, which allows to continuously adjust the laser energy to the individual requirements without beam displacement. This gradually coated filter is turned by a high precision DC stepping motor, which is conveniently regulated on the monitor screen by means of the PALM RoboSoftware (please refer to the PALM RoboSoftware Manual).

6.5.2 Laser Focus Adjustment

Precise laser focus alignment allows laser cutting with a nearly diffraction limited laser focus. The coincidence of the laser focus with the optical focus of the microscope is mandatory for precise laser micromanipulation.

Every wavelength is diffracted differently. This is important to know as the spectrum of the visible light of the microscope is at a different wavelength as the UV-laser. When performing laser micromanipulation at a greater distance from the specimen slide or within a thicker or strongly refracting object, the offset of the laser focus position has to be compensated. This is especially important for living cells. The same applies when using different objectives.

To compensate for the so called chromatic aberration the laser focus position has to be changed, independent from the microscope focus.

The Laser Focus Correction is done motorized: A high precision DC stepping motor moves the corresponding lens. Focus setting is conveniently controlled and regulated on the monitor screen by means of the of the PALM RoboSoftware. The display allows relocating selected laser focus settings.

Laser energy and focus is predefined by P.A.L.M. Microlaser Technologies GmbH over the factory settings for each objective (please refer to the PALM RoboSoftware Manual). Only the sample specific values have to be defined by the individual user. These user specific values can be stored in a special file for each user and/or application.

Notice

- The factory settings regarding the laser focus and energy settings have been adjusted to the objectives supplied by P.A.L.M. Microlaser Technologies GmbH (all of a comparable lens configuration). Please note: The factory settings apply to the standard P.A.L.M. membrane slide without any tissue.
- Please contact P.A.L.M. Microlaser Technologies GmbH prior to the use of other objectives.
- For the catapulting and microdissection, a LD 40x objective has proved successful in most of the applications.

For the visualization of catapulted specimen with the LD 40x objective, you need a cap with a flat, non-frosted clear surface and a rim, which is not higher than 2 mm (available from P.A.L.M. Microlaser Technologies GmbH).

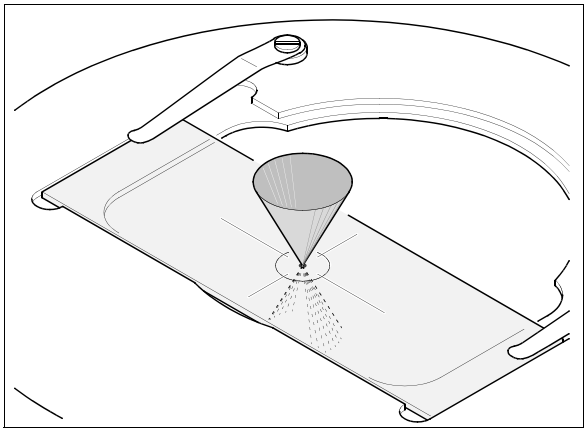


Figure 17 Laser focus in specimen plane

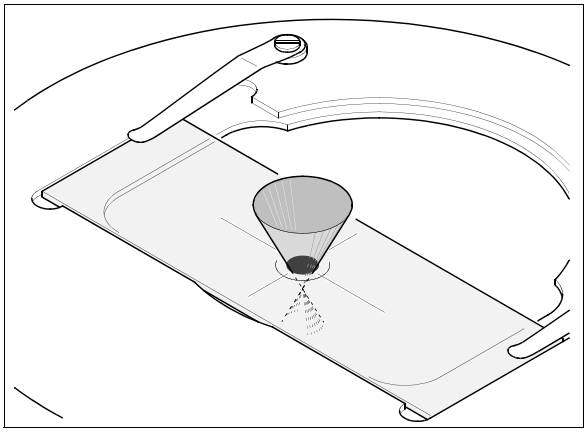


Figure 18 Laser focus below specimen plane

The smallest laser focus diameter can be obtained when using objectives of a high numerical aperture.

When using objectives with a lower numerical aperture, the minimal achievable diameter of the laser focus increases correspondingly.

When working with objectives having a short working distance, the thickness of the object slide must be adapted to the used objective. If the objective is manually adjustable, please assure that the objective is adjusted to the thickness of the actually used object slide.

6.6 How to adjust laser focus and energy

As the laser beam itself is not visible, only the laser effect (ablation) can be used for control.

To visualize the changes of the laser focus position, you must cut (ablate) an object, whereby the laser is permanently pulsating and the object is being moved, simultaneously. We recommend to use an LPC object slide (membrane-coated) for this purpose.

Notice
Make sure that the membrane is always focused within the microscope focus, otherwise the laser focus setting might be incorrect.

Simultaneous stage movement will create a cutting line within the sample. This line will be fuzzy or fairly thick due to yet insufficient laser focus setting.

A star-like glass ablation around the laser focus indicates a good focussing of the glass surface.

With a higher energy setting cutting is possible not only within the laser focus but also above or below. If the laser focus is just inside the glass, smaller or bigger pieces of glass will be blown out.

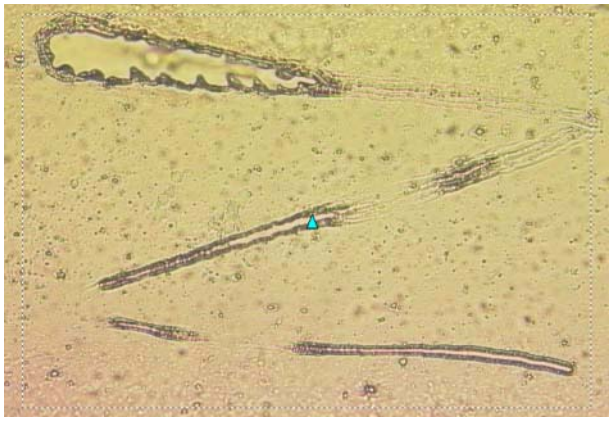


Figure 19 Zig-zag line cut with the laser with different energy and focus settings

Find the correct focal plane in the following ways (please refer to the PALM RoboSoftware Manual for using the graphic tools, the automated laser functions and how to adjust energy and focus settings for the laser):

- use a plain membrane-slide without tissue specimen
- draw a zig-zag line with the freehand graphic tool spanning at least the monitor screen
- choose an appropriate stage speed (not too fast)
- start automatic cutting with the "Cut function"
- reduce resp. increase the laser energy with the "Page down" resp. "Page up" key at the keyboard or with the slider for laser energy at the monitor screen until only a faint cut can be seen
- move the laser focus up and down with the keys "home" and "end" at the keyboard or the corresponding slider for laser focus at the monitor screen to locate smallest cutting size
- reduce the laser energy and repeat the above described procedure a second or third time, until the smallest available focal spot is found.

The perfect laser focus setting is achieved, when with a minimum of laser energy a fine cutting line is visible, which immediately disappears as soon as the laser focus is slightly shifted up or down.

The cutting edges have to show identical symmetry, when the object is moved into x- or into y-direction. With increasing energy the membrane margins should still appear symmetrically.

Notice
For each type of sample the laser focus adjustment has to be performed individually. The individually determined values can be stored in a separate file (please refer to the PALM RoboSoftware Manual).

Notice
For objectives with aperture diaphragm, adjust the diaphragm to the thickness of the object slide or the Petri dish used to ensure an optimum presentation (focusing, contrast).

For objectives with a short working distance special Petri dishes and 0.17mm slides are necessary.

6.7 Cutting speed

The laser pulse repetition rate of the N_2-laser is fixed at 30 pulses per second resp. 100 pulses per second for the FTSS Laser. Therefore, to reach a fine cutting line it is necessary to optimize the laser energy with regard to the cutting speed.

7 PALM MicroBeam with Fluorescence Illumination

7.1 Operate the Microscope with Fluorescence Illumination

The fluorescence attachment is mounted with clamping screws to the microscope adapter (please see Figure 8).

Notice
Make sure that there is no filter within the beam path of the laser that could block the laser transmission.

Therefore, the excitation filter wheel is mounted outside the laser beam path directly between the fluorescence lamp housing and the microscope adapter (see Figure 8, Pos. 1).

7.2 Simultaneous Fluorescence Observation

The beam splitter (reflector module in the reflector turret) is custom-made.

The beam splitter must have a special coating suitable for the simultaneous reflection of the laser light and the fluorescence excitation wavelength as well as for the transmission of the emitted fluorescence light.

Caution
Do not use at any rate the beam splitters offered by microscope manufacturers along with the laser because such beam splitters may be damaged by the high energy of the laser.

If you want to work with fluorescence and use the laser simultaneously you must use a special filter set available from P.A.L.M.

For further information please contact P.A.L.M. Microlaser Technologies GmbH.

7.3 Settings

7.3.1 Settings for working with motorized microscope (Zeiss Axiovert 200M)

All elements designed for the use under fluorescence illumination can be controlled by the PALM RoboSoftware. Basic settings are preloaded and can be changed and saved by the PALM RoboSoftware (please refer to the RoboSoftware manual).

In order to use the fluorescence illumination, you must switch on the fluorescence illumination and select the desired fluorescence filter unit.
With this the functions "(Excitation)filter", "Reflector position", "Microscope light", "Timer" and "Shutter" are performed via a single command.

The basic settings of these functions have been made during installation of your PALM system. The PALM RoboSoftware provides you with the capability of saving various combinations of these parameters and retrieving them when they are needed.

Note:
Close the shutter before switching on the fluorescence lamp and before finishing or interrupting your work. This will prevent unintentional exposure of your sample to fluorescence light.

7.3.2 Settings for working with manual microscope (Zeiss Axiovert 200)

In order to use the fluorescence illumination, you must

- switch on the fluorescence illumination
- select the desired fluorescence excitation filter (only necessary when using a filter wheel; please refer to the PALM RoboSoftware Manual)
- swivel in the desired reflector position in the microscope
- switch the microscope light on or off as necessary
- activate the timer (if you want to preset a time for which the excitation light should illuminate your sample; refer to the PALM RoboSoftware Manual), and
- open the shutter (please refer to the PALM RoboSoftware Manual).

The PALM RoboSoftware controls all functions of the filterwheel and the shutter (please refer to the PALM RoboSoftware Manual).

Settings of the microscope has do be done manually.

7.4 Freeze Mode

A very special feature is the possibility of "freezing" the fluorescence image on the screen with the PALM RoboSoftware tool "Freeze Mode" (please refer to the PALM RoboSoftware Manual). Thus bleaching effects of the sensitive samples can be avoided while defining the laser cutting lines is possible (e.g. freehand, select elements). In the "Freeze Mode" the fluorescence is deactivated as well as all functions to move the stage. After handling the "frozen" picture on the screen, you can leave the "Freeze Mode" to continue with other regions under fluorescence or carry out laser functions.

Note:
Before you switch on a laser function you have to turn the reflector revolver of the microscope into a position which is suitable coated for this wavelenght. For basic fluorescence attachments this is the first position only.

8 Trouble Shooting

In general, if malfunctions occur with the laser system, first ascertain that all connections to power supply and laser head, as well as to existing external or internal control and safety systems (Interlock, enable, key switch in position "1"), are plugged.

8.1 FTSS Laser

No.	Error	Possible Cause	Correction
1	The laser system switches off automatically during operation. The LED1 is yellow.	Out of the operating temperature range, e.g. the laser head is overheated.	Let laser system cool down and switch on again. Reset the control (e.g. by the reset switch on the front panel - left from LED2).
		Room temperature too high.	Check for the reason of the high temperature and make sure of a cooling down.
		Internal electrical fault in laser head or in control unit.	Contact customer service.
2	Red LED "Laser On" fails to light up even though the button On/ Off was actuated. LED3 fails to light up.	see No. 1.	see No. 1.
		INTERLOCK signal is missing.	Check if INTERLOCK connection on "Interlock" and the external control and safety systems are connected properly.
		The LED3 fails to light up, which means laser system is not ready for operation.	- Wait for 3 minutes until LED3 becomes green. - Laser diode driver is out of order. Switch off and on the power supply. Try to start the laser again.
		Internal electrical fault in laser head or in control device.	Contact customer service.
3	LED2 fails to light up even though the power switch was actuated.	Power supply is not activated.	Check input voltage and power supply cable. Check fuse beside the main switch.
		Control unit is defective.	Contact customer service.
4	LED3 is red but laser emission is absent.	External or internal trigger is not activated or does not work properly.	- Check external trigger pulses. - Disconnect external trigger and use the internal trigger to check the laser.
		Control unit or booster is defective.	Contact customer service.
5	No indication of LED2 and LED3.	Power supply is not activated.	- Check input voltage and power supply cable. - Check key switch position and fuse beside main switch (stand alone).
		LEDs are deactivated.	Hardware reset can reactivate the diodes. Reset the control (e.g. by the reset switch on the front panel - left from LED2).
		Control unit is defective.	Contact customer service.

8.2 Nitrogen Laser

check energy and focus settings as described in section 6.6, page 25, if necessary take factory settings		
check red LED on Laser unit		
	LED is on	o.k.
	LED is off	check key switch on laser unit
		check power connection of laser
check green LED on Laser unit		
	LED is on	o.k.
	LED is off	check function of safety switch in illumination arm of the microscope
check position of adjusting aid for the fluorescence lamp (see Figure 7, Pos. 9)		
	left position	o.k.
	right position	switch to left
check shutter for FL (see Figure 7, Pos. 3)		
	shutter open (FL on display)	o.k.
	shutter closed	open shutter (press FL on/off button)
check position of reflector turret		
	reflector turret on position "laser"	o.k.
	any other position (except you have fluorescence equipment with P.A.L.M. simultaneous cutting)	switch to "laser"
check both sliders with diaphragm (see Figure 7, Pos. 10)		
	diaphragm open	o.k.
check position of filter slider (see section 11, page 15)		
	one of the three open positions	o.k.
check position of the correction ring (if available) of the objective according to the thickness of the slide		

8.3 No video image

check image in oculars		
	no image:	check illumination of microscope
check position of main-switch on camera's power supply (if applicable)		
check connection between camera and camera power supply (if applicable)		
check connection between camera and computer; S-VHS-plug must be plugged in framegrabber board		
check position of switching mirror in microscope		

8.4 Shift between drawing and cutting

check position of laser marker		
check alignment of camera; the camera must be adjusted parallel to the stage movement, test this by moving the stage with the arrow keys (in a slow speed)		
Calibrate new as described in the PALM RoboSoftware Manual; make sure that you are using the settings for the active objective		
check LED's on encoder of moving stage:		
	LED is green:	o.k.
	LED is orange or red:	please contact P.A.L.M. Microlaser Technologies GmbH

8.5 Reflex in video image

Incidence of light through the ocular may cause reflexes in the video image		
	in case of reflexes	close shutter at the microscope

9 Maintenance of the PALM MicroBeam

Caution
Before starting any maintenance works, disconnect all system components from the mains.

9.1 Laser

See Laser Manual

9.2 Microscope

For detailed actions see Microscope Manual

9.2.1 Cleaning of the Optical Parts

- Clean all easily accessible optical parts once a week.
- Carefully wipe the optical parts with a cleaning tissue moistened with alcohol of 70 to 80 %.

9.2.2 Cleaning When Actually Dirty

Actual dirt, e. g. caused by finger prints or immersion oil, must immediately be removed, otherwise the optical functions will permanently be impaired.

Dirt on surfaces of lenses or filters burnt-in by laser light can not be removed any more.

- Slightly moisten a cleaning tissue with pure alcohol (100 %) or spirit and wipe the dirty parts very carefully.
- Clean dry objectives and oculars smoothly with a cotton bud or a tooth pick padded with cotton wool. Only use surgical cotton from the pharmacy.
- Remove cotton fluffs and similar by blowing with a small pair of bellows, such as an enema syringe.

Caution

Moisten cleaning tissue only slightly, do not soak. Excessive solvent might dissolve the cementing of the lenses.

Do not use acetone for cleaning, at any rate.

10 Technical Data

10.1 Electricity

Input voltage	115/230 V AC
Voltage fluctuations	± 5%
Frequency	50/60 Hz
Frequency fluctuations	± 2 Hz

10.2 Nitrogen Laser

Wavelength	337 nm
Pulse energy	>270 µJ
Pulse duration	3 nsec
Pulse frequency	1-30 pulses per second
Beam divergence	0.3 mrad
Laser control	Laser control can be changed by impulse packages: - external, by earth-free foot stepper or via RS232 interface - automatic, permanent impulses Impulse width: 0.1 ms
Pulse frequency of laser control	Adjustable via software

10.3 FTSS Laser

Wavelength	355 nm
Pulse energy	90 µJ
Pulse duration	1 nsec
Pulse frequency	100 pulses per second
Beam divergence	3 mrad
Laser control	Laser control can be changed by impulse packages: - external, by earth-free foot stepper or via RS232 interface - automatic, permanent impulses Impulse width: 0.1 ms
Pulse frequency of laser control	Adjustable via software

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